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Identification of Diagnostic Biomarkers, Immune Infiltration Characteristics, and Molecular Subtypes Based on Histamine-related Genes in Sepsis

Jun Zhang, Huijing Tong, Xiaojun Wang, Yingwei Ding, Linghong Xu, and Gang Chen,

Department of Emergency, Affiliated Jinhua Hospital, Zhejiang University School of Medicine, Jinhua, Zhejiang, China

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ABSTRACT

Sepsis is a life-threatening systemic inflammatory response syndrome marked by high mortality and immune dysfunction. Histamine, synthesized from histidine, by histidine decarboxylase (HDC), regulates immune cell recruitment and inflammatory mediators, playing a key role in inflammatory diseases. The precise mechanisms and clinical significance of histamine in sepsis require further study.

Gene expression data from the Gene Expression Omnibus (GEO) database were analyzed. Differential expression analysis and weighted gene co-expression network analysis (WGCNA) were used to identify differentially expressed histamine-related genes (DEHRGs). Machine learning algorithms, including the least absolute shrinkage and selection operator (LASSO), support vector machine-recursive feature elimination (SVM-RFE), and random forest (RF), were utilized to screen diagnostic genes, and a predictive model was constructed and validated using receiver operating characteristic analysis and decision curve analysis (DCA). Functional enrichment, immune infiltration assessment, using single-sample gene set enrichment analysis (ssGSEA), cell-type identification by estimating relative subsets of RNA transcripts (CIBERSORT), regulatory network construction, and drug prediction were subsequently conducted.

Nine DEHRGs were identified. Three key diagnostic genes-*FYN*, *IL2RB*, and *MMP8*-were selected and validated across multiple cohorts, showing high diagnostic accuracy (area under the curve [AUC]>0.85). The study revealed distinct immune patterns, including increased regulatory T cell (Treg) infiltration in the sepsis group. Two sepsis molecular subtypes with differential immune characteristics were also identified.

This study systematically explored the association between histamine and sepsis pathogenesis, defining a three-gene diagnostic model and elucidating complex immune and molecular regulatory mechanisms. These findings offer new insights for developing targeted diagnostic and therapeutic strategies for sepsis.

Keywords: Biomarkers; Histamine; Immune system phenomena; Molecular typing; Sepsis

Corresponding Author: Gang Chen, BM;
Department of Emergency, Jinhua Hospital Affiliated to Zhejiang University School of Medicine, Jinhua, Zhejiang, China. Tel: (+86 152) 6863 2203, Fax: (+86 152) 6863 2203, Email: cgang261@163.com

INTRODUCTION

Sepsis is a life-threatening condition associated with extremely high mortality among critically ill patients. It

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is driven by an abnormal systemic inflammatory response that triggers an excessive release of inflammatory mediators.[1] This cascade subsequently leads to widespread dysfunction across multiple organs, including the heart, liver, lungs, kidneys, and brain.[2] Epidemiological data indicate that sepsis affects millions of people worldwide each year, with a mortality rate as high as 30% to 50%.[3] Although advances have been made in resuscitation strategies, mechanical ventilation management, antibiotic therapy, and blood glucose normalization, no treatment beyond standard supportive care has proven particularly effective., and mortality remains high.[4,5] Therefore, an in-depth exploration of the biological mechanisms and potential biomarkers associated with sepsis is of great significance for reducing disease risk and improving patient prognosis.

Histamine is synthesized from histidine through the catalytic action of histidine decarboxylase (HDC) and is primarily produced and stored in mast cells, basophils, enterochromaffin cells, and several types of immune cells.[6] It functions both as a local mediator involved in inflammatory responses and as a neurotransmitter in physiological processes. Histamine exerts its diverse biological effects through four types of receptors (H1–H4): in the vascular system, it induces vasodilation and increases vascular permeability; in the immune system, it modulates the release of inflammatory mediators, leukocyte mobilization, and chemotaxis, thereby influencing the intensity of inflammatory responses.[7] In the pathophysiological process of sepsis, both clinical and experimental studies have indicated that elevated histamine levels or upregulation of histamine-related pathways correlate with disease severity and organ dysfunction.[8,9] Moreover, studies have shown that deletion of *HDC* or blockade of histamine receptors (such as H1/H2) can attenuate lung, liver, and kidney injury and improve survival in sepsis models, suggesting that the histamine pathway may contribute to the pathological progression of septic organ injury.[9,10] However, the precise role of histamine in sepsis remains unclear. Understanding the association between histamine and sepsis may facilitate the development of diagnostic and therapeutic strategies for this disease.

In this study, a comprehensive bioinformatics approach was employed to systematically investigate the relationship between histamine and the pathogenesis of sepsis. Using machine learning techniques, we developed a high-precision diagnostic model to identify key biomarkers, which demonstrated excellent discriminatory performance. This study not only provides new insights into the underlying mechanisms of sepsis but also offers a powerful tool for improving diagnosis and developing targeted therapeutic strategies.

MATERIALS AND METHODS

Data Acquisition and Processing

Gene expression microarray data for sepsis were downloaded from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). The GSE65682-training dataset, consisting of 93 samples (51 sepsis and 42 healthy), was used as the training set. Three independent datasets, GSE26378, GSE95233, and GSE65682-validation, were used as validation sets. The GSE26378 dataset includes 103 samples (82 sepsis and 21 healthy), while the GSE95233 dataset contains 124 samples (102 sepsis and 22 healthy). To ensure the robustness of our findings in a larger population, a large-scale cohort from GSE65682-validation, consisting of 802 samples (760 sepsis and 42 healthy, comprising a broad spectrum of infectious etiologies to test clinical robustness), was also included for validation. To minimize the impact of batch effects and ensure cross-study comparability, inter-array normalization was performed for each dataset using the *normalizeBetweenArrays* function within the *limma* R package. A total of 1581 histamine-related genes (HRGs) were obtained from the GeneCards database (https://www.genecards.org/).

Identification of DEHRGs Based on WGCNA and Differential Expression Analysis

Differential expression analysis between sepsis and healthy samples was performed using the limma package, with thresholds of adjusted p value less than 0.05 and absolute $\log_2$ fold change ($|\log_2(FC)|$) greater than 0.585. Weighted gene co-expression network analysis (WGCNA) was then conducted. Hierarchical clustering was performed on all genes. A soft threshold of $\beta = 14$ was chosen to group genes with similar expression patterns into co-expression modules, based on a scale-free topology fit of $R^2 = 0.9$. Module-trait relationships were assessed by calculating the Pearson correlation between each module and clinical traits. Genes within the module showing the highest correlation were defined as hub genes using thresholds of absolute module membership (|MM|) greater than 0.8

and gene significance (|GS|) greater than 0.4. Finally, the intersection of differentially expressed genes (DEGs), hub module genes, and HRGs was taken to identify differentially expressed histamine-related genes (DEHRGs).

Machine Learning and Diagnostic Model Construction

Machine learning algorithms were applied to identify key feature genes with potential diagnostic value. Specifically, least absolute shrinkage and selection operator (LASSO), support vector machine–recursive feature elimination (SVM-RFE), and random forest (RF) analyses were performed using the glmnet, e1071, and *randomForest* packages, respectively. For the LASSO analysis, a binomial logistic regression model was constructed with the parameter α=1, and a 10-fold cross-validation (n-fold=10) was utilized to identify the optimal penalty parameter (lambda), selecting genes at the minimum lambda (lambda.min). The SVM-RFE model was executed with 10-fold cross-validation to determine the feature subset that yielded the minimum classification error. For the RF algorithm, a 10-fold cross-validation was similarly applied to evaluate feature importance based on the Gini impurity index; genes exhibiting a Mean Decrease Gini score exceeding 2.0 were prioritized as candidate biomarkers. The intersections of genes identified by these three complementary algorithms were ultimately defined as the core feature genes. In the GSE65682-training dataset, ROC curve analysis was conducted for the key feature genes identified by machine learning using the pROC package. The area under the ROC curve (AUC) was utilized to evaluate the overall discriminatory power of the diagnostic model in distinguishing between sepsis patients and healthy controls. An AUC value typically ranges from 0.5 to 1.0, where a value closer to 1.0 signifies superior diagnostic performance. Genes with AUC > 0.7 were considered diagnostic genes and were used to construct a diagnostic model. A nomogram was generated using the rmda package, and corresponding calibration curves were plotted. Decision curve analysis (DCA) was performed to evaluate clinical benefit. Finally, a comprehensive comparative analysis evaluated the performance of our model against previously reported sepsis diagnostic models.

Gene Set Enrichment Analysis and Pathway Analysis

Gene set enrichment analysis (GSEA) was performed for the diagnostic genes using the clusterProfiler R package. All Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were also conducted using the clusterProfiler package.

Immune Infiltration Analysis

Immune infiltration analysis evaluated different cell types and functional features. Single-sample GSEA (ssGSEA) calculated immune infiltration scores, while cell-type identification by estimating relative subsets of RNA transcripts (CIBERSORT) analysis characterized differences in immune cell infiltration. Statistical comparisons of immune infiltration levels were conducted between healthy and disease groups. Pearson correlation analysis was performed between infiltrating immune cells and diagnostic genes. Additionally, immune infiltration analysis was performed using the Microenvironment Cell Populations-counter (MCP-Counter) algorithm implemented in the IOBR R package.

Construction of Potential TF and miRNA Target Gene Regulatory Networks and Prediction of Small Molecule Drugs

The miRNet (https://www.mirnet.ca/) online database identified potential microRNAs (miRs) targeting the diagnostic genes, visualized with Cytoscape. NetworkAnalyst (https://www.networkanalyst.ca/) predicted upstream transcription factors (TFs). The drug signature database (DSigDB, http://dsigdb.tanlab.org/DSigDBv1.0/) evaluated potential protein-drug interactions by leveraging the "Enrichr" suite (https://maayanlab.cloud/modEnrichr/) (Supplementary Table 1).

Subtype Identification of Diagnostic Genes

Subtype identification of sepsis patient samples in the GSE65682-training dataset was performed using the non-negative matrix factorization (NMF) algorithm implemented in the `NMF` R package, based on the diagnostic genes obtained from the model. Differential expression analysis between subtypes was conducted with thresholds of adjusted $p<0.05$ and $|\log_2(FC)|>1$ to characterize subtype-specific profiles.

Statistical Analysis

All analyses were performed using R (version 4.4.0).

The Wilcoxon test determined statistical differences between two groups. Data were primarily visualized using the ggplot2 package. The Pearson method evaluated associations between variables. Statistical significance was determined at a p value less than 0.05.

RESULTS

Analysis of DEHRGs in Sepsis Patients

Differential expression analysis identified 800 DEGs (436 upregulated and 364 downregulated) (Figure 1A). Key module analysis using WGCNA with a soft threshold of $\beta = 14$ (Figure 1B) identified 4 co-expression modules (Figure 1C). The turquoise module significantly negatively correlated with sepsis ($p<0.001$, $r = -0.75$) and positively with the healthy group ($p<0.001$, $r = 0.75$) (Figure 1D). A total of 69 genes in the turquoise module were hub module genes. Correlation analysis revealed a significant positive relationship between MM and GS ($r = 0.55$, $p<1.7\times10^{-37}$) (Figure 1E). GO enrichment of hub genes showed significant involvement in antigen processing, T cell activation, and antibacterial defense (Figure 1F). KEGG results showed enrichment in autoimmunity and viral infection pathways (Figure 1G). The intersection of DEGs, hub genes, and HRGs yielded 9 DEHRGs (Figure 1H). GO enrichment of DEHRGs demonstrated significant involvement in immune regulation, secretion, and platelet function (Figure 1I).

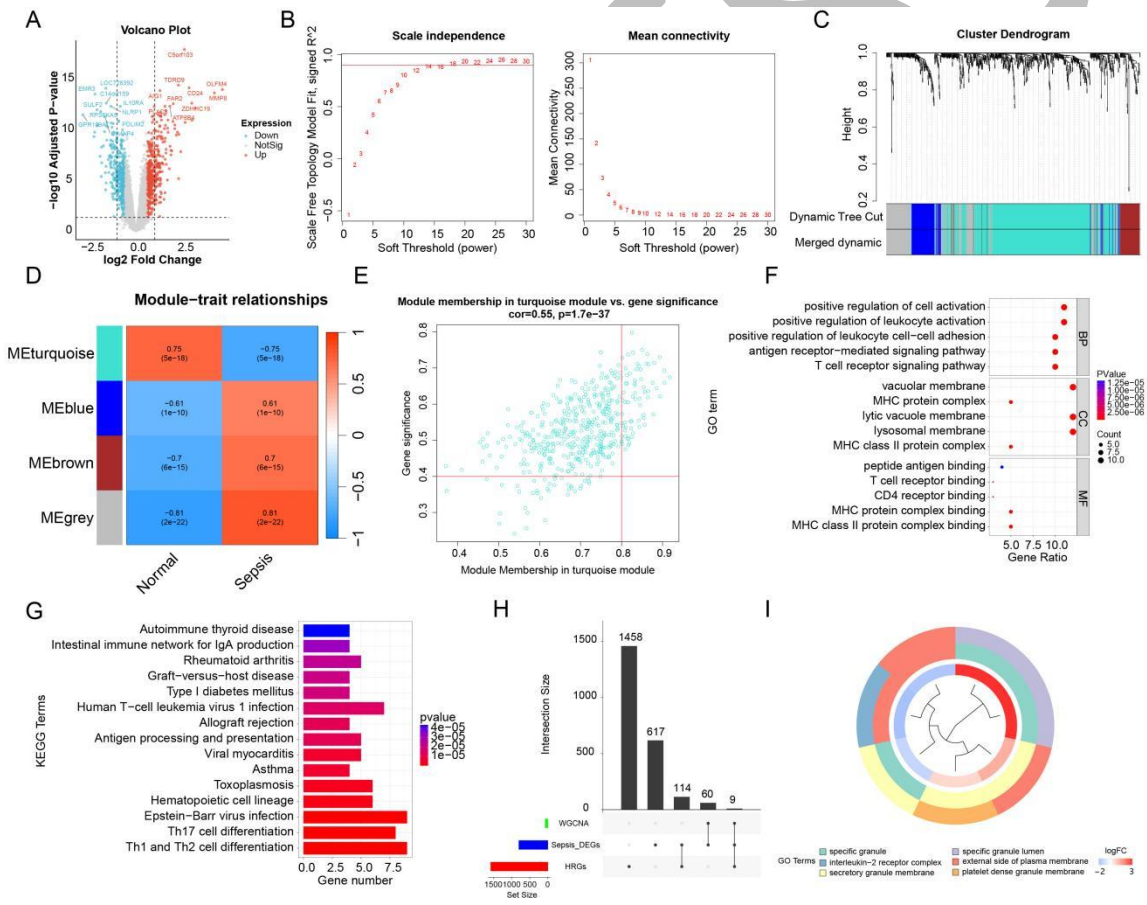

Figure 1. Identification and functional annotation of DEHRGs in sepsis

Identifying Important Variables with Machine Learning

Machine learning algorithms (LASSO, SVM-RFE, and RF) selected 6, 6, and 8 important variables, respectively (Figures 2A–E). The intersection of these identified 3 core feature genes: *FYN*, *IL2RB*, and *MMP8* (Figure 2F). Expression levels of *FYN* and *IL2RB* were significantly higher in sepsis compared to normal,

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whereas *MMP8* was significantly lower (Figure 2G). GSEA indicated that *FYN* and *IL2RB* were enriched in translation and immune-related processes (Figures 2H–I), while *MMP8* was enriched in T cell–mediated immunity (Figure 2J).

Diagnostic Model Construction and Validation

Individual feature genes demonstrated superior discriminatory capacity. Specifically, AUC values for *MMP8*, *IL2RB*, and *FYN* were 0.912 (95% CI: 0.8533–0.9716), 0.893 (95% CI: 0.8319–0.9543), and 0.892 (95% CI: 0.8233–0.9600), respectively. The integrated diagnostic model achieved an AUC of 0.933 (95% CI: 0.8824–0.9832) (Figure 3A). A nomogram illustrated the contribution of these 3 genes (Figure 3B). DCA indicated significant clinical net benefit within a risk threshold range of 0.1–0.6 (Figure 3C). Calibration curves showed predictions were highly consistent with the ideal reference (Figure 3D). Validation results confirmed high accuracy: GSE26378 (AUC=0.990, Figure 3E), GSE95233 (AUC=1.000, Figure 3F), and the larger GSE65682-training cohort (AUC=0.997, Figure 3G). Benchmarked against established models, our signature exhibited enhanced performance (Figure 3H, Supplemental Table 2).

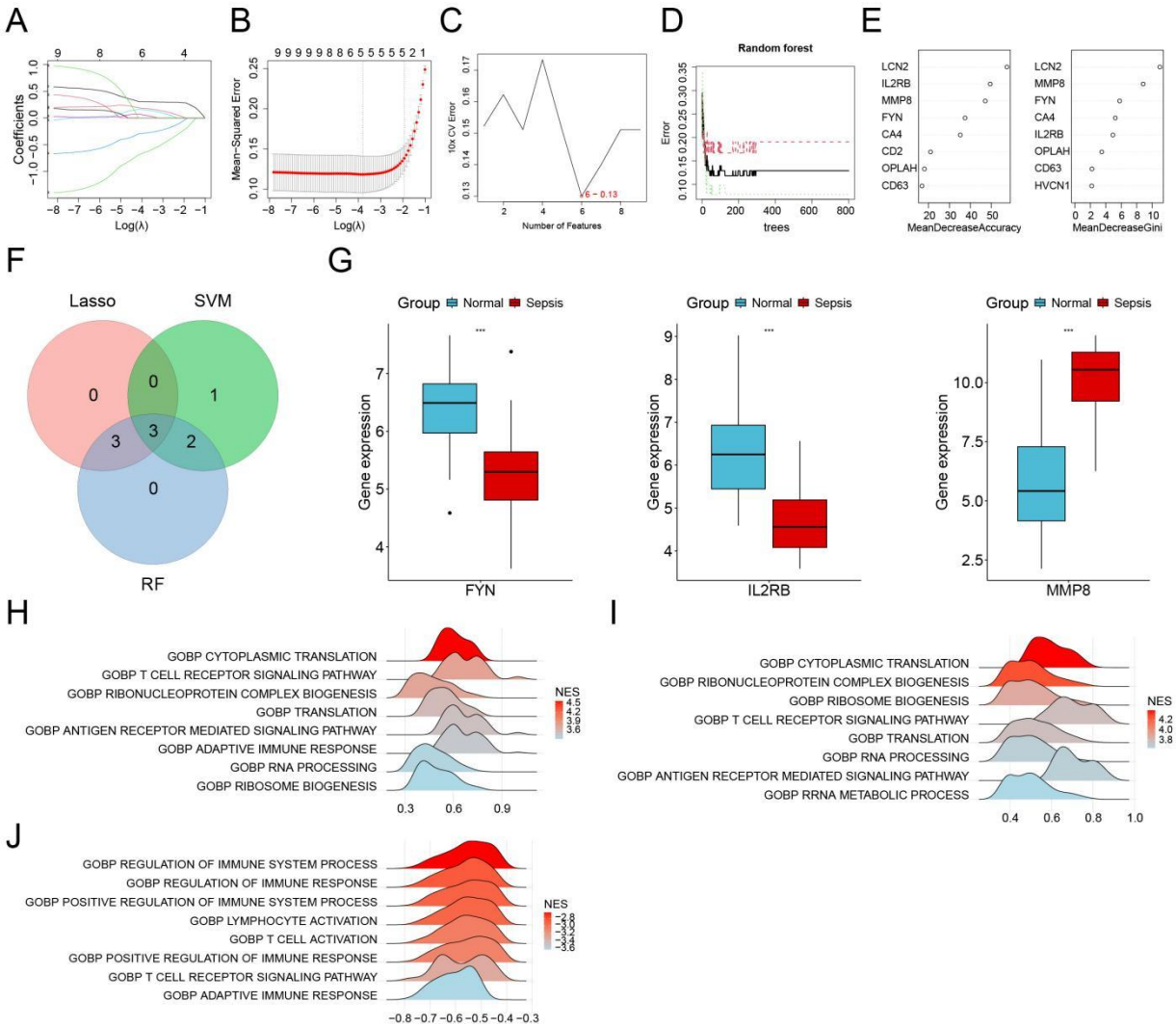

Figure 2. Multi-algorithmic screening and characterization of diagnostic genes

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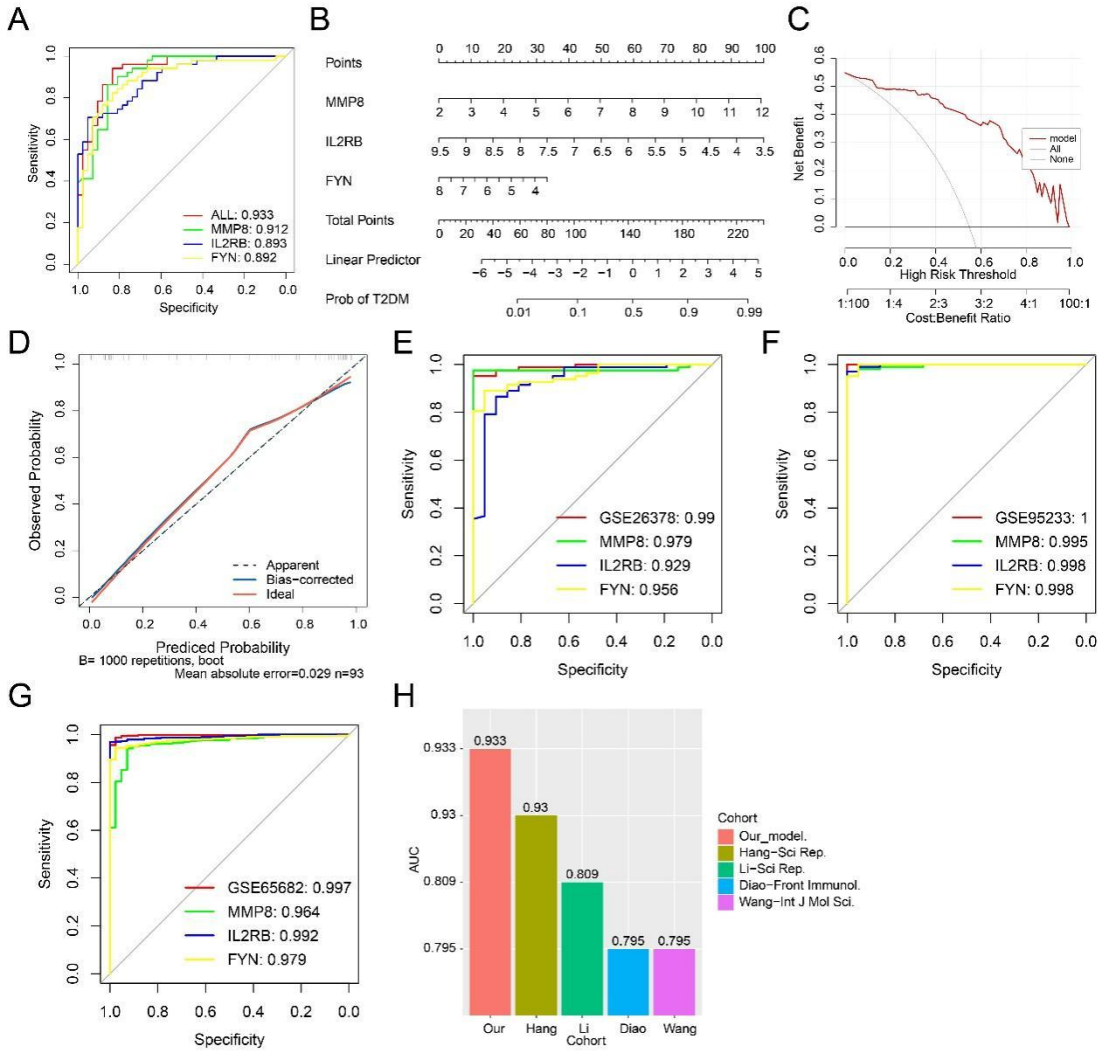

Figure 3. Development and validation of the three-gene diagnostic model

Immune-related Analysis

ssGSEA showed increased infiltration of $CD8^+$ T cells, neutrophils, NK cells, pDCs, Tfh, T_H2 cells, and TIL in the healthy group, whereas sepsis showed increased regulatory T cell (Treg) infiltration (Figure 4A). Immune function scores were generally higher in the healthy group, while Type II IFN response scores were elevated in sepsis (Figure 4A). CIBERSORT analysis revealed the healthy group had increased monocytes, whereas sepsis showed elevated plasma cells, M0 macrophages, and resting mast cells (Figure 4B). MCP-Counter indicated higher T cells, $CD8^+$ T cells, cytotoxic lymphocytes, NK cells, and neutrophils in the healthy group, while sepsis showed higher endothelial cells (Figure 4C). Biomarker expression significantly correlated with various immune cell infiltration levels (Figures 4D–F).

Construction of Potential TFs and miRNA Regulatory Networks and Therapeutic Targeting Prediction

Transcription factors, including *YY1*, *EGR1*, *CREB1*, and *ZNF354C*, were identified as key regulators interacting with multiple diagnostic genes (Figure 5A). The miRNA regulatory network revealed complex miR-mediated regulation of these genes (Figure 5B). Drug prediction from DSigDB identified phorbol 12-myristate 13-acetate, ilomastat, Ro-4396686, CGS-27023A, and CHEMBL475540 as potential therapeutic agents (Table 1).

Subtype Identification of Diagnostic Genes

NMF analysis determined the optimal number of clusters as *K*=2 (Figures 6A–B). Principal component analysis (PCA) demonstrated clear separation between Subtype 1 and Subtype 2 (Figure 6C). *FYN* and *IL2RB* were upregulated in Subtype 1, while *MMP8* was upregulated in Subtype 2 (Figure 6D). Subtype 1 showed significantly higher immune infiltration and function scores (Figure 6E). CIBERSORT revealed increased resting NK cells and M0 macrophages in Subtype 2, while neutrophil infiltration was elevated in Subtype 1 (Figure 6F). Differential analysis identified 122 DEGs between subtypes (Figure 6G), significantly enriched in immune and inflammatory responses (Figure 6H).

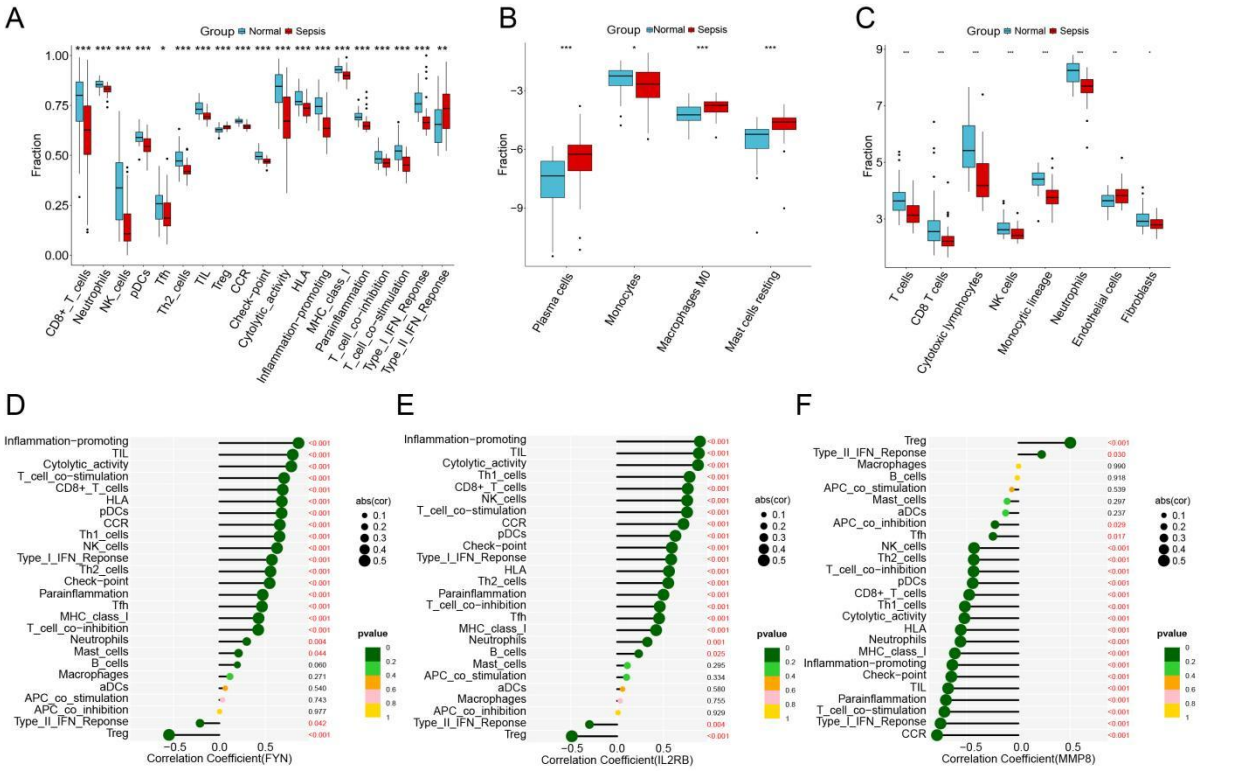

Figure 4 shows characterization of the immune microenvironment.

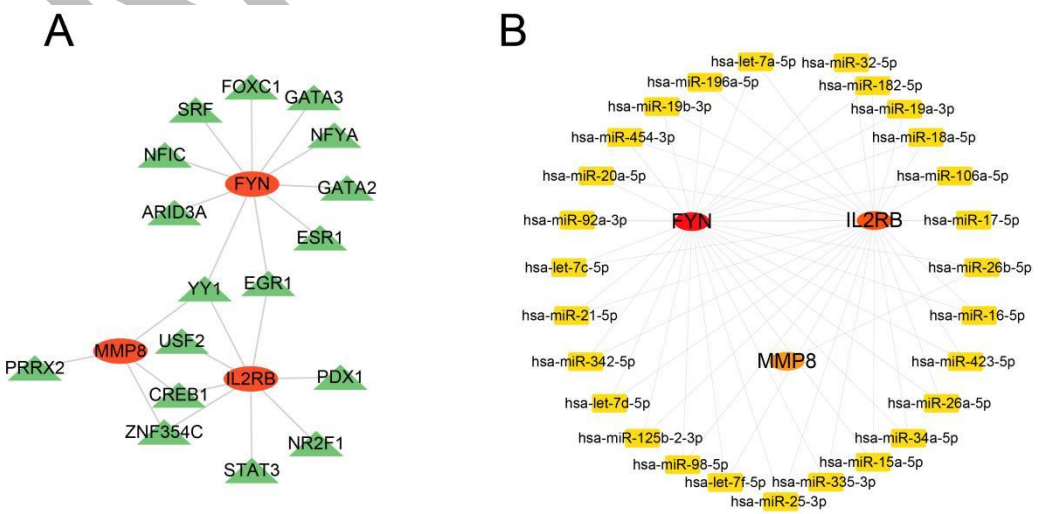

Figure 5. Construction of regulatory networks

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Table 1. Drug prediction for diagnostic genes

Term	*p*	Adjusted *p*	Odds Ratio	Combined Score	Genes
Phorbol 12-myristate 13-acetate CTD 00006852	0.001718	0.038636	81.14761	516.6292	*IL2RB*;*MMP8*
Ilomastat TTD 00008545	0.001799	0.038636	908.4545	5741.921	*MMP8*
Ro-4396686 TTD 00010666	0.001799	0.038636	908.4545	5741.921	*FYN*
CGS-27023A TTD 00002801	0.001949	0.038636	832.7083	5196.554	*MMP8*
CHEMBL475540 TTD 00006054	0.002099	0.038636	768.6154	4739.656	*MMP8*

CTD: Comparative Toxicogenomics Database; FYN: FYN proto-oncogene, Src family tyrosine kinase; IL2RB: interleukin 2 receptor subunit beta; MMP8: matrix metallopeptidase 8; TTD: Therapeutic Target Database.

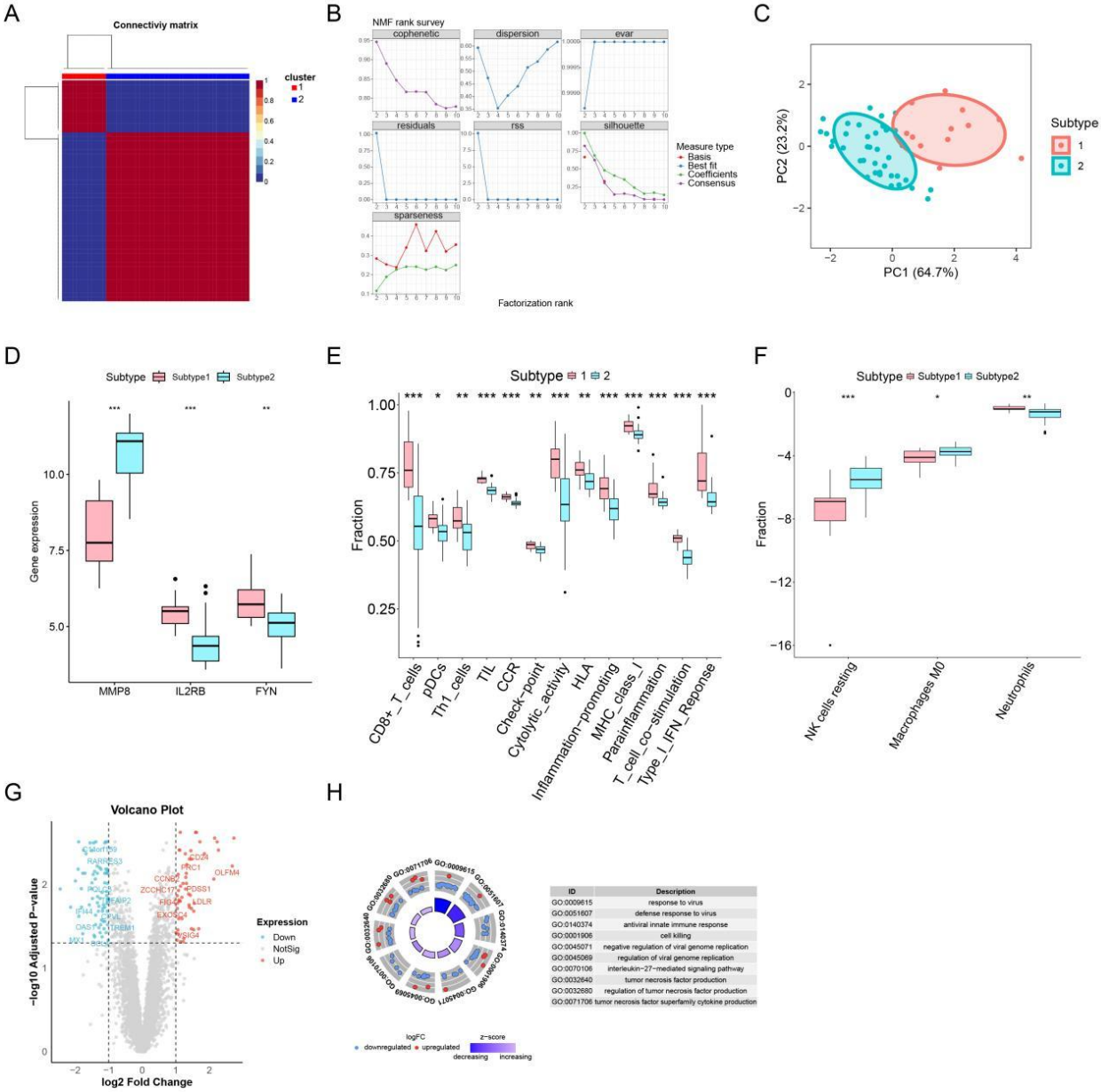

Figure 6. Identification of sepsis molecular subtypes

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DISCUSSION

In this study, multiple bioinformatics approaches were applied to systematically identify and validate histamine-related diagnostic biomarkers in sepsis. Through bulk transcriptomic analysis, we characterized the histamine-related landscape of sepsis and established a robust diagnostic model centered on 3 key genes (*MMP8*, *IL2RB*, and *FYN*). These genes not only exhibited strong discriminatory power in distinguishing sepsis patients from controls but were also closely associated with immune infiltration patterns and potential regulatory mechanisms involving transcription factors and miRs. Furthermore, non-negative matrix factorization (NMF) analysis revealed 2 distinct sepsis subtypes with different immune and molecular characteristics, highlighting the heterogeneity of the disease. Collectively, these findings underscore the crucial role of histamine in the pathogenesis of sepsis and provide new insights into its diagnostic and therapeutic targeting.

Histamine, a pivotal mediator in the pathophysiology of sepsis, drives immune dysregulation and multi-organ dysfunction through intricate molecular pathways. Regarding immune imbalance, histamine significantly suppresses the production of pro-inflammatory cytokines, such as IL-12 and TNF-α, while concurrently stimulating the secretion of anti-inflammatory mediators like IL-10 via H2 receptors, thereby inducing a T_H1-to-T_H2 immune polarization.[11] This persistent immune shift leads to a profound state of immunosuppression, which severely impairs the host's capacity to clear secondary infections—a critical determinant of the high mortality rates observed in the late stages of sepsis.[12] In terms of organ dysfunction, histamine-mediated disruption of the endothelial barrier serves as a central mechanism for sepsis-induced multi-organ failure. Histamine activates H1 receptors on vascular endothelial cells, triggering downstream signaling cascades-including p38 MAPK, myosin light chain kinase, and the Rho/ROCK pathway-which collectively promote the formation of intercellular gaps and a rapid increase in vascular permeability.[13] Such systemic vascular leakage constitutes the pathological foundation for sepsis-associated acute lung injury and acute kidney injury.[9] Furthermore, pharmacological blockade of histamine receptors has been demonstrated to effectively attenuate organ damage and enhance survival in sepsis models, further underscoring the indispensable role of histamine in sepsis progression.[10]

The 3 diagnostic genes identified in this study play crucial roles in the pathogenesis of sepsis. *MMP8* (Matrix Metalloproteinase-8), also known as neutrophil collagenase or collagenase-2, is primarily secreted by neutrophils.[14] It is a neutral protease that mainly degrades collagen, and its expression in sepsis is closely associated with disease severity and prognosis.[15] *MMP8* is upregulated under sepsis conditions, and its genetic knockout or pharmacological inhibition has been shown to improve survival and alter inflammatory responses in mouse models of sepsis, suggesting that this gene contributes not only to extracellular matrix degradation but also to the modulation of inflammatory signaling, such as the NF-κB pathway.[16,17] Moreover, elevated plasma *MMP8* levels have been correlated with mortality and early disease progression, supporting its potential as a prognostic biomarker for sepsis,[18] which is consistent with our findings. *IL2RB* (Interleukin-2 receptor beta) is an essential component of the IL-2/IL-15 receptor complex, which governs the survival and functional balance of T cells, NK cells, and regulatory T cells.[19] In sepsis, changes in *IL2RB* expression are associated with immune dysfunction and altered cytokine production.[20] Studies have shown that modulation of *IL2RB* by miRs, such as miR-497-5p, can alleviate sepsis-induced acute lung injury, highlighting its key regulatory role in sepsis-associated immune dysregulation and organ damage.[21] *FYN*, a member of the Src family kinases, acts as a central regulator in multiple signaling pathways involved in cell proliferation, differentiation, adhesion, and migration.[22] Specifically, *FYN* participates in the development and activation of T lymphocytes and NK cells.[23] It also regulates platelet function by modulating signaling cascades such as PI3K and MAPK, thereby influencing platelet shape change, granule secretion, and adhesion.[24] Several studies have identified *FYN* as a key gene or potential biomarker in sepsis, and its differential expression among immune cell subsets suggests that *FYN* may contribute to sepsis pathogenesis by modulating adaptive immunity and intercellular signaling.[25,26] The 3 diagnostic genes (*MMP8*, *IL2RB*, and *FYN*) may synergistically promote the pathogenesis of sepsis by enhancing inflammatory responses and disrupting immune homeostasis.

The GSEA results for the three core biomarkers

provide deeper mechanistic insights into the divergent immunological landscapes observed in sepsis patients. For *FYN* and *IL2RB*, we observed a significant positive enrichment in pathways such as "T cell receptor signaling pathway", "Adaptive immune response" and "Cytoplasmic translation". *FYN*, as a member of the Src family kinases, is indispensable for initiating T-cell receptor signaling,[27] while *IL2RB* is a key driver of lymphocyte proliferation and differentiation.[28] The concurrent enrichment of these adaptive immune pathways and translational machinery underscores that high expression of *FYN* and *IL2RB* represents a state of robust immune mobilization and protein synthesis, likely reflecting an effective early-phase defense or a persistent attempt at pathogen clearance. In stark contrast, *MMP8* exhibited a prominent negative enrichment in pathways including "T cell activation", "Lymphocyte activation" and "Adaptive immune response". As *MMP8* is primarily released by neutrophils during degranulation, its high expression is strongly linked to systemic inflammation, endothelial barrier disruption, and organ injury.[13,16] The negative association between *MMP8* and adaptive immune pathways suggests that as neutrophil-mediated innate responses become hyper-activated and pathogenic, there is a concomitant suppression of lymphocyte-mediated adaptive immunity.[29] This "immune dissociation" characterizes the progression toward immunosuppression, where the host suffers from both excessive tissue damage and a severely compromised ability to mount a specific immune response, a critical determinant of late-stage sepsis mortality.[30] These findings further confirm that *FYN*, *IL2RB*, and *MMP8* are not merely diagnostic markers but are central functional executors of the immune dysregulation that defines sepsis.

In the control group, multiple effector immune cells, including $CD8^+$ T cells, neutrophils, and NK cells, exhibited increased infiltration along with activated proinflammatory pathways, indicating an overall enhanced immune activation profile. In contrast, the sepsis group exhibited upregulation of inhibitory immune signals. Notably, Treg cells were markedly increased in the sepsis group, and previous studies have demonstrated that Tregs can directly suppress effector T-cell activation by secreting inhibitory cytokines such as IL-10 and TGF-β.[31] This phenomenon reflects the typical immunosuppressive state observed in the later stages of sepsis, during which the immune system restrains excessive inflammation through Treg expansion and other inhibitory mechanisms, such as the accumulation of myeloid-derived suppressor cells.[12,32] Concurrently, the significantly reduced infiltration of neutrophils observed in the sepsis group does not signify an attenuation of the inflammatory response; instead, it reflects a phenotypic transition toward a pathogenic state. Evidence suggests that the efficient eradication of pathogens is fundamental to improving sepsis prognosis, a process in which neutrophils play a cornerstone role.[33] Nevertheless, during sepsis, the inhibition of glycolysis has been shown to induce neutrophil immunosuppression,[29] leading to compromised antimicrobial activity and subsequently dysregulated immune responses.

The regulatory network established in the current study identifies several potential molecular candidates for the targeted treatment of sepsis. On one hand, *YY1* and *EGR1* were identified as core TFs that bridge multiple diagnostic genes. It has been reported that *YY1* expression in the peripheral blood mononuclear cells of pediatric sepsis patients is negatively correlated with cell apoptosis; furthermore, diminished *YY1* levels are linked to multiple organ dysfunction and poor clinical outcomes,[34] suggesting that *YY1* may influence the progression and prognosis of sepsis by modulating apoptotic processes. In addition, *EGR1* has been confirmed to play a vital role in sepsis-associated signaling pathways. Recent mechanistic evidence indicates that the stabilization of *EGR1*, mediated by *PRMT1*, exacerbates sepsis-associated acute lung injury, implying that therapeutic interventions targeting *EGR1* or its upstream regulatory components could serve as a strategy to mitigate systemic organ injury.[35] On the other hand, miR-21-5p emerges as a significant player within the miRNA regulatory network in sepsis. In patients with polytrauma who subsequently develop sepsis, exosomal miR-21-5p levels are significantly downregulated and exhibit a strong correlation with sepsis incidence, underscoring its potential as a predictive biomarker for disease onset.[36] Furthermore, research has demonstrated that miR-21-5p can inhibit the progression of lipopolysaccharide-induced sepsis by downregulating *PDCD4*, thereby mitigating damage to cardiomyocytes and other vital tissues.[37]

The identification of two sepsis-related subtypes through NMF in this study underscores the profound heterogeneity in immune status and prognostic risk among sepsis patients. Subtype 1, characterized as an "immune-activated" phenotype, is defined by the robust

expression of *FYN* and *IL2RB*. Consistently, ssGSEA results indicate a significant enrichment of pro-inflammatory signatures in this subtype, including $CD8^+$ T cells, T_H1 cells, and Type I IFN response. Given that *IL2RB* is a pivotal receptor for sustaining T-cell activity[19] and *FYN* is essential for early T-cell receptor signaling,[23] patients in Subtype 1 may represent the early hyper-inflammatory phase of sepsis or exhibit a superior capacity for pathogen clearance. Conversely, Subtype 2 manifests an "immunosuppressive and high-injury-risk" profile, marked by significantly elevated *MMP8* levels alongside diminished *FYN* and *IL2RB* expression. CIBERSORT analysis reveals an increased infiltration of M0 macrophages and resting NK cells in this subtype, suggesting that these patients may reside in an immunosuppressive state with compromised cytotoxic functions. As *MMP8* levels correlate positively with disease severity, organ injury, and increased mortality,[16] Subtype 2 patients are likely predisposed to a higher risk of multiple organ dysfunction syndrome and generally poorer clinical outcomes. Based on the distinct expression patterns of *FYN*, *IL2RB*, and *MMP8* between these subtypes, these genes emerge as potential biomarkers for rapid clinical stratification, thereby facilitating the development of personalized and stratified immunotherapeutic strategies.

In this study, an integrative bioinformatics approach was employed to identify histamine-related biomarkers and elucidate the potential immune landscape. Although our study successfully established a three-gene diagnostic model and revealed the roles of specific immune cells such as Treg cells and neutrophils, several limitations remain. The roles of the three identified diagnostic biomarkers in sepsis are predicated on transcriptomic predictions rather than direct clinical trial validation. Future research should prioritize validating the differential expression of these biomarkers at both mRNA and protein levels using techniques such as qRT-PCR, Western blotting, and immunohistochemistry in independent patient cohorts and specimens. Furthermore, in vitro functional assays are warranted to elucidate their specific contributions to inflammatory signaling, immunosuppression, and pathogen clearance. Regarding the immune infiltration analysis, we utilized a synergistic approach combining ssGSEA and CIBERSORT. The strength of ssGSEA lies in its capacity to calculate immune functional scores, while CIBERSORT offers higher precision in resolving relative immune cell proportions. Additionally, the relatively limited sample size presents a potential risk of overfitting. While we implemented strategies like normalization and cross-validation, external validation remains a priority. Potential confounders, including age, sex, and underlying comorbidities, may also influence transcriptomic profiles. We addressed this by validating our findings across multiple independent datasets; the robust performance suggests generalizability across distinct patient populations. Future research should integrate multiomics data from large-scale cohorts with cellular and molecular experiments to systematically unveil clinical translational value.

In summary, this study systematically explored the relationship between histamine and the pathogenesis of sepsis by integrating bulk RNA sequencing data. We successfully established a gene-based diagnostic model for sepsis and conducted an in-depth investigation of the complex molecular mechanisms involved, encompassing immune infiltration, TF and miRNA target-gene regulatory networks, and drug prediction. These findings provide new insights into the pathophysiology of sepsis and lay a foundation for the development of histamine-based diagnostic approaches and targeted therapeutic strategies in the future.

STATEMENT OF ETHICS

Not applicable.

FUNDING

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

Not applicable.

DATA AVAILABILITY

The data and materials in the current study are available from the corresponding author on reasonable request.

AI ASSISTANCE DISCLOSURE

Not applicable.

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